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BRIEF REPORT

Emergence of Panton-Valentine leukocidin-positive ST59 methicillin-susceptible *Staphylococcus aureus* with high cytolytic peptide expression in association with community-acquired pediatric osteomyelitis complicated by pulmonary embolism

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antibiotic treatment;

A 15-year-old boy, who had had a furuncle on his femur, developed femoral pyomyositis and osteomyelitis complicated by septic pulmonary embolism. Panton-Valentine leukocidin-positive (PVL^+) ST59 methicillin-susceptible *Staphylococcus aureus* (MSSA) was isolated from pus

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community-associated methicillin-susceptible *Staphylococcus aureus* (CA-MSSA);
genotype;
pediatric osteomyelitis;
septic pulmonary embolism

and blood. Chemotherapy was started with cefazolin, followed by combination therapy with meropenem/vancomycin with surgery. The MSSA (strain KS1) was positive for increased levels of cytolytic peptide (*psmα* and *hld*) and staphylococcal enterotoxin B (SEB), and manifested IS*1216V*-mediated multidrug resistance (to erythromycin, clindamycin, kanamycin, streptomycin, and chloramphenicol), similar to a genome-analyzed reference strain (PM1) of ST59/SCC*mec*V(5C2&5) community-associated methicillin-resistant *S. aureus* (Taiwan CA-MRSA), but unlike another reference strain (M013) of Taiwan CA-MRSA in terms of resistance. The data suggest that CA-MSSA KS1, characterized by PVL, increased levels of cytolytic peptide, SEB, and multidrug resistance, is a possible ancestral strain of Taiwan CA-MRSA and causes the unique association of osteomyelitis and septic pulmonary embolism, requiring complicated management.
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Introduction

Although methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen, known as healthcare-associated MRSA (HA-MRSA), MRSA has also been isolated in the community since 1997—1999 and is known as community-associated MRSA (CA-MRSA).[1] CA-MRSA exhibits increased expression of cytolytic peptide [or phenol-soluble modulin (PSM)] genes and often produces Panton-Valentine leukocidin (PVL).[2,3] The most characterized PVL-positive (PVL^+) CA-MRSA includes four clones: multilocus sequence type 8 (ST8)/staphylococcal cassette chromosome *mec* type IV (SCC*mec*IV), known as USA300; ST30/SCC*mec*IV; ST80/SCC*mec*IV; and ST59/SCC*mec*V, known as the Taiwan clone.[3,4] Other characteristics unique to each clone are the arginine catabolic mobile element (ACME) for USA300[3]; and for the Taiwan CA-MRSA clone, staphylococcal enterotoxin B (SEB) and multidrug resistance (to erythromycin, clindamycin, kanamycin, streptomycin, chloramphenicol, and tetracycline), encoded by the enterococcal IS*1216V*-associated mobile element structure (MES_{PM1}) and MES_{tet}.[4,5]

Osteomyelitis is an invasive infection mostly caused by *S. aureus*, and with inappropriate treatment, it can be a devastating or even fatal disease.[6] In the United States, where USA300 is the predominant CA-MRSA,[3] MRSA osteomyelitis cases, occasionally complicated with septic pulmonary embolism, have increased.[7,8] Moreover, USA300-type CA-MSSA, which lacks SCC*mec*, accounts for a growing proportion (around 25%) of CA-MSSA and is associated with increased cases of pediatric invasive CA-MSSA infections, especially osteomyelitis.[8] Here, we describe the unique association of osteomyelitis and septic pulmonary embolism due to PVL^+ CA-MSSA related to the Taiwan CA-MRSA clone, which required complicated management.

Case Report

A 15-year-old boy (baseball player) was admitted due to fever and pain with swelling of the left femur in August 2010. He developed a furuncle accompanied by pus discharge twice on the anterior surface of the left knee after an insect bite, but showed improvement 15 days before admission. On admission, body temperature was 39.0°C, white blood cell (WBC) count was 18,600/μL, platelet count was 185,000/μL, C-reactive protein (CRP) was 16.8 mg/dL, and the erythrocyte sedimentation rate (ESR) was 67 mm/hour. Pyomyositis in the vastus intermedius and abscesses in the subperiosteal area were noted on the left lower thigh, and also inflammation spreading to the bone marrow (osteomyelitis) was suspected on magnetic resonance imaging (MRI, Fig. 1A). Incision and pus drainage were performed, and the administration of cefazolin (3 g/day) was initiated. Cultures of blood and pus, obtained from the subperiosteal area of the femur, revealed PVL^+ MSSA. In spite of cefazolin administration at high doses, dyspnea gradually developed on Day 5, with continuous hyperthermia ($\geq$ 39°C) and with no improvement of his laboratory data (CRP, 15.6 mg/dL; WBC, 8300/μL). A diagnosis of septic pulmonary embolism was made on Day 5, based on contrast-enhanced computer-assisted tomography (CT) findings (Fig. 1B). A CT scan also showed posterior pleural and pulmonary infiltrates (Fig. 1B), suggesting pneumonia. Cefazolin was changed to meropenem (2 g/day) according to the Japanese guidelines for hospital-acquired pneumonia,[9] because the possibility that his respiratory disturbance was caused by pneumonia could not be ruled out. On Day 8, hyperthermia ($\geq$ 38°C) continued despite some improvement of his laboratory data (CRP, 9.8 mg/dL; ESR, 88 mm/hour; WBC, 13,500/μL). Left femoral osteomyelitis was still noted, and femoral fenestration and drainage were performed. Vancomycin (1 g/day) was added for possible MRSA infection. Dyspnea improved on Day 10 and clinically improved on Day 15: body temperature, 36.8°C; CRP, 0.3 mg/dL; ESR, 30 mm/hour; WBC count, 4800/μL; and platelet count, 256,000/μL. Based on clinical effectiveness, the administration of meropenem (for a further 2 weeks) and vancomycin (for a further 3 weeks) was continued; due to his unstable temperature (occasionally $>$ 37°C), this was followed by oral cloxacillin for 6 weeks. He was discharged on Day 76. No MRSA was isolated throughout his clinical course.

The pus MSSA isolate was designated as KS1 and molecular characterizations were performed as described previously.[5,10,11] Briefly, multilocus sequence typing (MLST) was performed using seven housekeeping genes, and an allelic profile (allele no.; ST type) was obtained from the MLST website (http://www.mlst.net/). The *spa* type was analyzed using a polymerase chain reaction (PCR) [targeting the *spa* (protein A) gene], and determined using a public *spa*-type

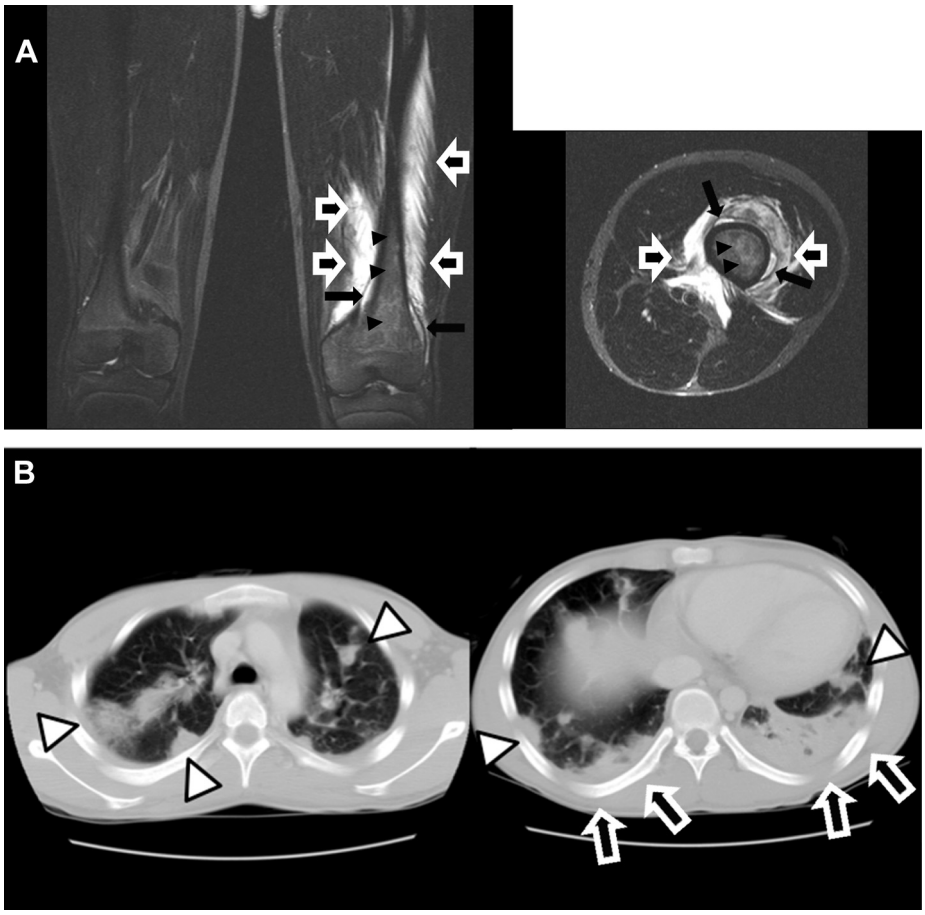

Figure 1. Left femoral osteomyelitis complicated by septic pulmonary embolism in a 15-year-old boy (baseball player). In (A), showing lower limb magnetic resonance imaging (MRI), high-intensity regions were noted in the (intermediate vastus) muscle around the distal end of the left femur on fat-suppressed T2-weighted imaging (pyomyositis, large arrows; subperiosteal abscess, small arrows), and the bone marrow was accompanied by high-intensity regions and weak enhancement (inflammation of the bone, arrowheads). In (B), chest computer-assisted tomography (CT) showed bilateral multiple foci of consolidations (septic embolism, arrows), with pleural effusion and pulmonary infiltrate (pneumonia, arrowheads) at the posterior.

database (http://tools.egenomics.com/) or Ridom SpaServer (http://spaserver.ridom.de/). Typing of *agr* was carried out by PCR and the *agr*-variable region was sequenced as previously described.[12] Coagulase (Coa) typing was conducted using a staphylococcal Coa antiserum kit (Denka Seiken, Tokyo, Japan). Virulence genes were analyzed by PCR[11,13]; the target genes in PCR included 50 genes: three leukocidin genes (*luk$_{PV}$SF*, *lukE-lukD*, and *lukM*), five hemolysin genes (*hla*, *hlb*, *hlg*, *hlg-v*, and *hld*), a peptide cytolysin (*psm*α), 19 SE genes (*tst*, *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*, *sep*, *seq*, *ser*, and *set*), one putative staphylococcal enterotoxin gene (*seu*), three exfoliative toxin genes (*eta*, *etb*, and *etd*), a staphylococcal superantigen-like gene cluster (*ssl*), the epidermal cell differentiation inhibitor gene (*edin*), 14 adhesin genes (*icaA*, *icaD*, *eno*, *fib*, *fnbA*, *fnbB*, *ebpS*, *clfA*, *clfB*, *sdrC*, *sdrD*, *sdrE*, *cna*, and *bbp*), the staphylokinase gene (*sak*) carried by the innate immune evasion cluster (IEC) of phage 3 (φSA3), and the ACME-*arcA* gene. The amount of SEB in the supernatant of bacterial cultures at $2.0 \times l0^9$ CFU/mL was examined using a SET-RPLA kit (Denka Seiken) according to the instructions of the manufacturer. For pulsed-field gel electrophoresis (PFGE), bacterial DNA was digested with *Sma*I, and the digested DNA was electrophoresed in 1.2% agarose with marker DNA (Lambda ladder; Bio-Rad Laboratories, Inc., Hercules, CA, USA) as described previously.[12] DNA banding patterns of MSSA KS1 were compared with those of eight strains of PVL$^+$ ST59/SCC*mec*V CA-MRSA (Taiwan clone), which included seven strains from Taiwan (PM1, PM2, PM5, PM6, PM11, PM17, and TSGH17) and one strain (OS7) from Japan[10,12]; and also with the banding patterns of two strains of PVL-negative (PVL$^-$) ST59/SCC*mec*IV CA-MRSA, which included PM34 and PM36 from Taiwan.[10] Susceptibility testing of bacterial strains was carried out using the agar dilution method with Mueller-Hinton agar.[14] Antimicrobial agent interactions were evaluated by agar checkerboard tests; the fractional inhibitory concentrations (FIC) of ≤ 0.5, > 0.5—4.0, and > 4.0 were defined as synergy, no interaction (nonsynergic), and antagonism.[15] Drug resistance genes, *mecA* (encoding for resistance to β-lactam agents including methicillin, oxacillin, and cephems), *ermB* (encoding for erythromycin and clindamycin resistance), *aph(3′)-IIIa* (encoding for kanamycin resistance), *aadE* (encoding for streptomycin resistance), and *cat* (encoding for chloramphenicol resistance), were analyzed as previously described.[5,10] The mRNA expression levels of the

cytolytic peptide (PSMα and Hld) genes (*psmα* and *hld*) and 16S rRNA genes were examined by reverse transcription (RT)-PCR assay.[16] The *psmα* and *hld* expression levels were normalized by the 16S rRNA expression level. The level of significance was defined as $p < 0.05$.

Strain KS1 was susceptible to oxacillin [minimum inhibitory concentration (MIC), 0.13 μg/mL], and negative for the *mecA* gene in PCR. KS1 was also susceptible to cefazolin, meropenem, and vancomycin; MIC values (μg/mL) were 0.25, 0.03, and 1.0, respectively. The *in vitro* effect of meropenem/vancomycin combination against KS1 showed no interaction (nonsynergistic), but had an FIC of > 0.5 to 1.0.

Molecular characteristics of KS1, compared with genome-analyzed reference strains (PM1 and M013) of the Taiwan CA-MRSA clone, are summarized in Table 1. KS1 belongs to the genotype (ST59/*spa*143[t437]/*agr*1a/Coa VII) and carries the genes for PVL, four hemolysins (α-hemolysin, Hla; β-hemolysin, Hlb; γ-hemolysin, Hlg; δ-hemolysin, Hld), three staphylococcal enterotoxins (SEB, SEK, SEQ), and 11 adhesins (*icaA*, *icaD*, *eno*, *fib*, *fnbA*, *fnbB*, *ebpS*, *clfA*, *clfB*, *sdrC*, and *sdrE*), similar to the Taiwan CA-MRSA clone, including strain PM1.[5,10] The SEB production levels of KS1 and PM1 were 15.6—31.3 μg/mL. Moreover, similar to PM1, KS1 was resistant to five non-β-lactam agents, erythromycin (MIC, ≥ 256 μg/mL) and clindamycin (MIC, ≥ 256 μg/mL), kanamycin (MIC, ≥ 256 μg/mL), streptomycin (MIC, ≥ 256 μg/mL), and chloramphenicol (MIC, 64—128 μg/mL), and had each corresponding drug resistance gene, *ermB*, *aph(3′)-IIIa*, *aadE*, and *cat*.

Since strain PM1 (Taiwan CA-MRSA clone) carries the five non-β-lactam resistance genes in a unique MES (MES_{PM1}), with the array of IS*1216V*-*ermB*-*aph(3')-IIIa*-*aadE*-IS*1216V*-IS*1216V*-*cat*-IS*1216V*-IS*1216V*,[5] KS1 was investigated for the presence of the MES_{PM1} structure by PCR and sequencing. As expected, KS1 also possessed the same MES_{PM1} structure, which was inserted into the chromosomal *sasK* gene with a 8-bp attachment sequence (*att*, consisting of tattaaat), similar to PM1 (Fig. 2A).

The genetic relatedness of KS1 and Taiwanese CA-MRSA was also determined by PFGE analysis (Fig. 2B). KS1 and strains of the Taiwan CA-MRSA clone (PVL^+ ST59/SCC*mec*V) comprised subclusters, in which each strain differed from others by no more than three bands, indicating a single clone (Taiwan CA-MRSA/MSSA clone). These subclusters of the Taiwan CA-MRSA/MSSA clone were divergent from those of PVL^- ST59/SCC*mec*IV CA-MRSA, another representative CA-MRSA in Taiwan[4,19] (Fig. 2B).

The expression levels of the cytolytic peptide genes (*psmα* and *hld*) are summarized in Fig. 2C. Regarding ST59 CA-MRSA, both PVL^+/SCC*mec*V and PVL^-/SCC*mec*IV (irrespective of PVL^+ and PVL^- types) expressed the *psmα* and *hld* genes at high levels, similar to USA300, but significantly higher than HA-MRSA, ST5/SCCmecII (New York/Japan clone) or ST239/SCCmecIII ($p < 0.05$). Unexpectedly, the expression levels of one Taiwan clone strain (TSGH17)[10,24] were very low; the reason remains unclear.

PVL^+ CA-MSSA strain KS1 expressed the *psmα* and *hld* genes at high levels, similar to CA-MRSA. When other MSSA

Table 1 Relevant characteristics of methicillin-susceptible *Staphylococcus aureus* (MSSA) strain KS1, compared with Taiwan ST59/SCC*mec*V CA-methicillin-resistant *S. aureus* (MRSA) reference strains PM1 and M013, whose genomes were analyzed

Type, virulence gene, drug resistance gene, genetic structure, phenotype, mutation	MSSA	Taiwan ST59/SCC*mec*V CA-MRSA	
	Strain KS1 (PCR/sequence data, etc.)	Strain PM1 (genome data[a], etc.)	Strain M013 (genome data[b])
Type			
ST	59	59	59
spa	143 (t437)	143 (t437)	143 (t437)
agr	1a[c]	1a[c]	1a[c,d]
SCC*mec* type	—	V (5C2&5) [e]	V (5C2&5) [d,e]
Coagulase type	VII	VII	VII[d]
Virulence gene			
Leukocidin genes			
$luk_{PV}SF$ (PVL)	+	+ ($\varphi SA2_{PM1}$) [f]	+ (phage) [f]
lukE-lukD	—	—	—
Hemolysins			
hla, *hlb*, *hlg*, *hlg-v*, *hld*	+	+	+
Enterotoxin			
SaPI3 (*seb1*, *sek*, *seq*)	+ (SEB, 16-32 μg/ml) [g]	+ (SEB, 16-32 μg/ml) [g]	+
tst, *sea*, *sec*, *sed*, *see*, *seg*, *see*, *sei*, *sej*, *sel*, *sem*, *sen*, *seo*, *sep* [h], *set*, *seu*	—	—	—
Exfoliative toxin			
eta, *etb*, *etd*	—	—	—
Others			
psmα	+ (high expression)	+ (high expression)	+ (expression, ND[i])
ssl	+	+	+

Table 1 (*continued*)

Type, virulence gene, drug resistance gene, genetic structure, phenotype, mutation	MSSA	Taiwan ST59/SCC*mec*V CA-MRSA	
	Strain KS1 (PCR/sequence data, etc.)	Strain PM1 (genome data[a], etc.)	Strain M013 (genome data[b])
edin	−	−	−
sak [h]	−	−	−
Adhesin			
c11ag[j]	+	+	+ (*fnbA*:167-bp insertion, resulting in frame shift) (*sdrC*: 24-bp deletion)
sdrD, *cna*, *bbp*	−	−	−
ACME (*arcA*)	−	−	−
Drug resistance			
β-lactam resistance			
mecA (MIC of oxacillin, μg/ml)	− (0.125)	+ (8)	+ (ND[i])
blaZ (ABPC$^{r/s}$)/PCase plasmid (size, kb)	- (ABPC^{s})/−[k]	+ (ABPC^{r})/pPM1 (26 kb) [l]	ND[i]/ND[i]
Non-β-lactam resistance			
MES_{PM1} (EM^{r}, CLDM^{r}, KM^{r}, SM^{r}, CP^{r}) [m]	+	+	−
MES_{tet} (TC^{r}) [l]	−	+[l]	ND[i]
Other phage			
$\Phi SA1_{PM1}$	ND[i]	+	−
Relevant mutation in another chromosomal gene			
sasA	ND[i]	−	54-bp deletion

[a] Data are from a previous paper[5] and GenBank Accession numbers ABFA01000000, AB699881, AB699882.
[b] Data are from a previous paper [17] and GenBank Accession number CP003166.
[c] *agr*1a, a variant of *agr*1.[12]
[d] Assigned, based on the reported sequences; a previous paper [17] and GenBank Accession number CP003166.
[e] SCC*mec*V (5C2&5), a composite type of SCC*mec*V; although SCC*mec*V (5C2) consists of type 5 *ccr* (*ccrC*) and class C2 *mec* gene complexes, SCC*mec*V (5C2&5) has an additional *ccrC* gene complex (= type 5 *ccr* gene complex).[18] The entire SCC*mec*V (5C2&5) sequences of strains PM1 and M013 show high homology (99.8%).
[f] PVL phage is described in parentheses. For strain M013, the name of the PVL phage is not described; a previous paper [17] and GenBank Accession number CP003166. The entire PVL phage sequences of strains PM1 and M013 are nearly identical (homology, > 99.9%).
[g] SEB production levels *in vitro*.
[h] Two genes (*sep* and *sak*) are located in the innate immune evasion cluster (IEC) of phage 3 (φSA3), which is present in PVL- ST59/SCC*mec*IV CA-MRSA (in Taiwan) but absent in PVL+ ST59/SCC*mec*V CA-MRSA (Taiwan clone).[5,17,19]
[i] ND, not determined or not described.
[j] *c11ag*, core 11 adhesin genes shared by all strains: *icaA*, *icaD* (for biofilm formation); *eno* (for laminin-adhesin); *fnbA*, *fnbB* (for fibronectin-adhesin); *ebpS* (for elastin-adhesin); *clfA*, *clfB*, *fib*, *sdrC*, *sdrE* (for fibrinogen-adhesin).
[k] Strain KS1 carries no plasmid.
[l] PCase plasmid (pPM1) of strain PM1 carries the 6-kb MES_{tet} structure, encoding for TC resistance.[5]
[m] A multidrug resistance structure (MES_{PM1}), encoding for resistance to EM, CLDM, KM, SM and CP, is located within the *sasK* gene on the chromosome (in strain PM1),[5] as shown in Fig. 2A; the same structure was also found in strain KS1.
ABPC = ampicillin; ACME = arginine catabolic mobile element; CLDM = clindamycin; CP = chloramphenicol; EM = erythromycin; KM = kanamycin; MES = mobile element structure; MIC = minimum inhibitory concentration; PCase = penicillinase; PVL = Panton-Valentine leukocidin; r = resistant; s = susceptible; SaPI = staphylococcal pathogenicity island; SCC*mec* = staphylococcal cassette chromosome *mec*; SEB = staphylococcal enterotoxin B; SM = streptomycin; ST = sequence type; TC = tetracycline.

strains were examined, the *psmα* expression levels of PVL+ MSSA strains isolated in the community were significantly higher than PVL- MSSA strains isolated in hospitals (Fig. 2C).

Discussion

Osteomyelitis is an infectious disease of the bones, predominantly from *S. aureus*, and is most often seen in children and elderly people. In pediatric *S. aureus* osteomyelitis, venous thrombosis occurs with or without the development of septic pulmonary embolism, which results in dyspnea with hyperthermia, albeit rarely.[7]

In the United States, USA300 is a major cause of the increase of osteomyelitis and also of venous thrombosis with septic pulmonary embolism,[7,8] and is associated with pathologic fracture following osteomyelitis.[25] With the spread of USA300, pediatric osteomyelitis cases from USA300-type MSSA have also increased.[8,26] In Taiwan, pediatric cases of bone/joint infections from CA-MRSA,

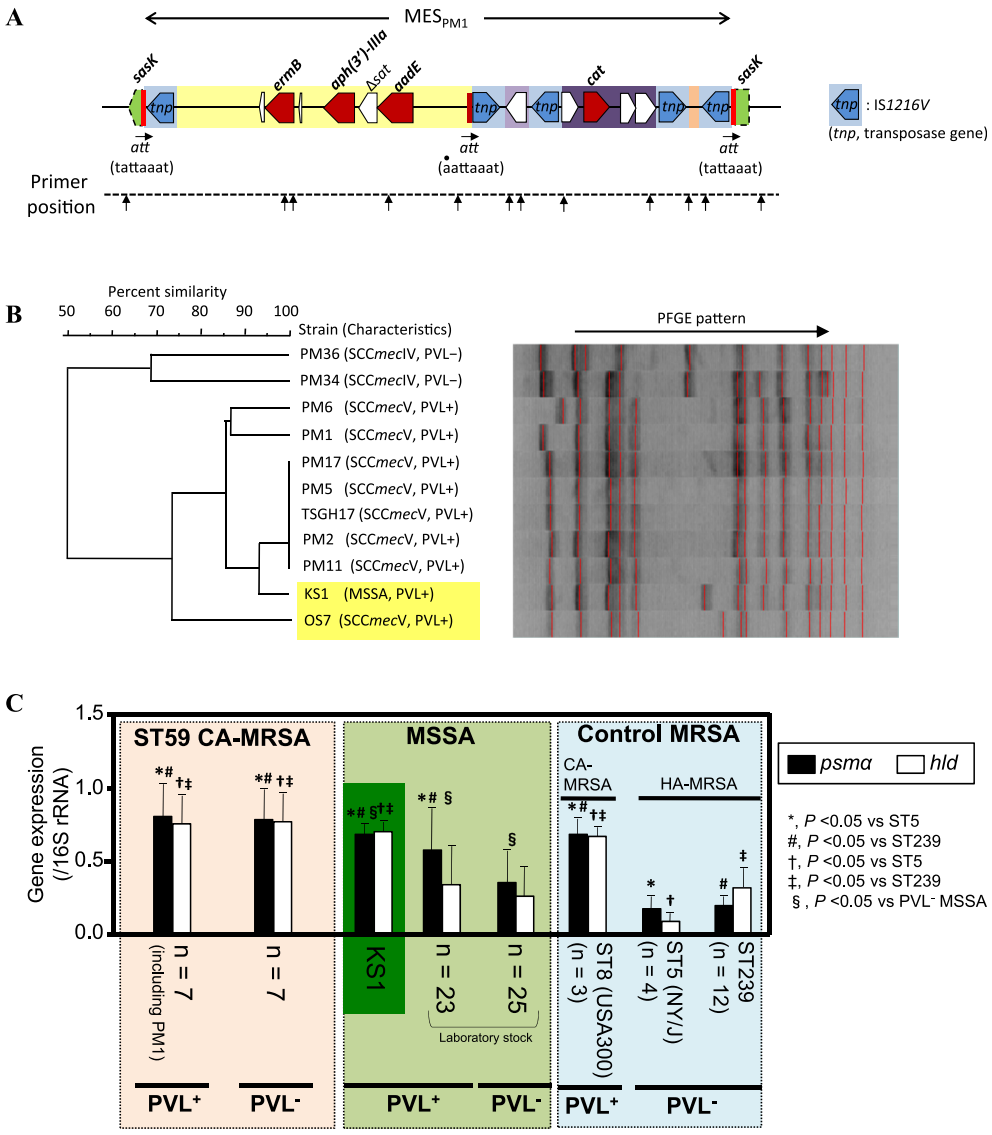

Figure 2. IS*1216V*-rich multidrug resistance structure (A), pulsed-field gel electrophoresis (PFGE) patterns (B), and mRNA expression levels of the cytolytic peptide genes (*psmα* and *hld*) (C) of Panton-Valentine leukocidin-positive (PVL$^+$) ST59 CA-methicillin-susceptible *Staphylococcus aureus* (MSSA) strain KS1. In (A), the structure of enterococcal IS*1216V*-mediated composite transposon (named MES$_{PM1}$) of the Taiwan clone [PVL$^+$ ST59/SCC*mec*V CA-methicillin-resistant *S. aureus* (MRSA) strain PM1] is shown at the top. The MES$_{PM1}$ is 21,832 bp long, flanked by direct repeats of IS*1216V* at both ends, and inserted into the *att* site (8-bp target sequence, tattaaat) within the *sasK* gene; the MES$_{PM1}$ contains three *att* sites (shown as a vertical red line).[5] The genes, *ermB*, *aph(3')-IIIa*, *aadE*, and *cat*, respectively, encode for resistance to erythromycin/clindamycin, kanamycin, streptomycin, and chloramphenicol. Vertical arrows below the MES$_{PM1}$ show the position of polymerase chain reaction (PCR) primers[5] used to identify the corresponding region (and sequence) of strain KS1; PCR and sequence analysis revealed that KS1 possesses exactly the same mobile element structure (MES) (MES$_{PM1}$) on the chromosome with the same *att* sequence. In (B), the PFGE pattern of KS1 was compared with the Taiwan CA-MRSA clone (strains PM1, PM2, PM5, PM6, PM11, PM17, TSGH17, and OS7)[10,12] and Taiwanese PVL$^-$ ST59/SCC*mec*IV CA-MRSA (strains PM34 and PM36).[10] Isolates from Japan (OS7 and KS1) are marked in yellow. In (C), the blue box shows the mRNA expression levels of two control MRSA groups, PVL$^+$ CA-MRSA (which shows high expression levels) and PVL$^-$ HA-MRSA (which shows low expression levels). Strains: (for ST8/SCC*mec*IV CA-MRSA, USA300) type strain USA300-0114, Japanese isolates NN36[20] and NN47[21]; [for ST5/SCC*mec*II HA-MRSA, New York/Japan (NY/J) clone] reference strains N315 and Mu50, strain I6 from Japan,[22] strain PM29 from Taiwan[10]; (for ST239/SCC*mec*III HA-MRSA) reference strains HU25 and ANS46, Taiwanese strains PM3, PM14, PM27, and PM38,[10] Russian strains 6K, 8K, 14K, 16K, 20K, and 51K.[23] The orange box shows the data of two predominant CA-MRSA in Taiwan. Strains: (for PVL$^+$ ST59/SCC*mec*V MRSA, Taiwan clone) PM1, PM2, PM5, PM6, PM11, PM17, and TSGH17 from Taiwan,[10] OS7 from Japan[12] (TSGH17 with unexpectedly low expression levels was omitted); (for PVL$^-$ ST59/SCC*mec*IV MRSA) PM34, PM36, PM42, and SSF17,[10] laboratory stock strains 4578, 6272-2, and 8038 from Taiwan. The green box shows the data of PVL$^+$ and PVL$^-$ MSSA. Deep green, KS1. Strains: (for PVL$^+$ laboratory stock MSSA) ST25, ST30, ST50, and ST121 isolates mostly from skin soft tissue infections, such as furuncles and bullous impetigo in the community; (for PVL$^-$ laboratory stock MSSA) isolates from blood, sputum, urinary tract discharge, and others of inpatients (probably also of outpatients).

including those with septic pulmonary embolism, have been reported[19,27,28]; all secondary pneumonia cases, preceded by bone/joint infections, accompanied septic pulmonary embolism and were considered to be caused by the Taiwan clone (PVL$^+$ ST59/SCC*mec*V) or its variant.[27] This study demonstrates the unique association of osteomyelitis and septic pulmonary embolism from PVL$^+$ ST59 CA-MSSA.

The patient was a young athlete (baseball player) at risk of community-acquired infection,[4] and suffered from skin and soft-tissue infection (SSTI, furuncle) prior to osteomyelitis. His family members (37-year-old father, 39-year-old mother, 13-year-old brother, and < 1-year-old sister) were all negative for PVL$^+$ MSSA and he had never been to Taiwan; therefore, the origin of his MSSA infection remains unexplained. Pyomyositis, subperiosteal abscesses, and osteomyelitis occurred on the left femur (the most common site of infection),[6,29] and the development of septic pulmonary embolism was an event related to bone infection.[7] After discharge, he suffered from a fracture at the same bone site as osteomyelitis. These clinical data suggest that KS1 is a highly virulent strain.

Osteomyelitis requires prolonged chemotherapy,[29,30] at least 4–6 or 8 weeks for MRSA infection[6,30] and approximately 3 weeks for MSSA infection,[6] until normalization of, for example, CRP or ESR levels.[6,29,30] For osteomyelitis, chemotherapy starts almost always empirically, and in children, empirical treatment may start with a first-generation cephem (cephalosporin), if the prevalence of MSSA in the community is > 90%; with clindamycin, if the prevalence of MRSA in the community is ≥ 10% and the prevalence of clindamycin-resistant *S. aureus* is < 10%; or with vancomycin, if the prevalence of MRSA and clindamycin-resistant *S. aureus* in the community is ≥ 10%[6]; the cutoffs of prevalence provided here are the personal opinion of some authors, not conclusions from well-designed trials.

Moreover, a protocol of safe switching from intravenous to oral medication has been discussed positively.[6] In a clinical observation in Taiwan, linezolid was used as step-down (oral) therapy for pediatric osteomyelitis due to MRSA (and MSSA), producing useful and well-tolerated results.[31] It has also been reported that clindamycin and linezolid, protein-synthesis inhibitors, suppress the production of some *S. aureus* toxins such as PVL *in vitro*.[32] Another report, however, did not recommend any specific antibiotic for PVL$^+$ cases.[33]

In this case, the initial therapy with cefazolin, a first-generation cephalosporin, was switched to intravenous combination therapy with meropenem and vancomycin with surgery, resulting in normalization of CRP after 9 days in total. The reason for cefazolin's inefficiency remains uncertain. However, since for osteomyelitis, the response to treatment is usually slow, in this case with an abscess in deep-seated structures, surgical debridement with a prolonged course of antibiotic treatment (even with cefazolin) could have been adequate.

Meropenem/vancomycin combination exhibits *in vitro* synergic (FIC ≤ 0.5) or additive (FIC > 0.5–1) action against ST5/SCC*mec*II HA-MRSA (New York/Japan clone).[34] In this study, the combination effect was synergic for ST239/SCC*mec*III, a dominant HA-MRSA clone in Taiwan (10 Taiwanese strains previously described[10]). However, the combination effect showed no interaction (nonsynergic), but had an FIC of > 0.5–1, against ST59 MSSA and MRSA, including KS1 (and other 9 MSSA strains), ST59/SCC*mec*V CA-MRSA (20 strains, including those used in Fig. 2C), and ST59/SCC*mec*IV CA-MRSA (10 strains, including those used in Fig. 2C).

Two strains of the Taiwan clone (PM1[5] and M013[17]) have been analyzed at genome levels, and the characteristics of KS1 were compared with those of reference strains. Regarding the SCC*mec* type of the Taiwan clone, initially, it was reported as type V_T, based on the partial SCC*mec* sequence of strain TGSH17[24]; later it was reported as SCC*mec*VII,[10,35] and finally reclassified as type V (5C2&5),[18] based on the entire SCC*mec* sequence of strain PM1, and more importantly based on the finding that the SCC*mec* was a composite SCC*mec* carrying two *ccrC* genes.[10,35]

The Taiwan clone has been multidrug-resistant to five non-β-lactam agents (erythromycin, clindamycin, kanamycin, streptomycin, and chloramphenicol), since its initial isolation in Taiwan,[4,5,10,24,36] unlike other major PVL$^+$ CA-MRSA.[4] Non-β-lactam resistance was encoded for by a unique mobile genetic structure MES_{PM1}, which was rich in IS*1216V*[5]; recombination events between IS*1216V* copies within the structure could result in various drug resistance patterns.[5]

IS*1216V* originates in enterococci.[37] IS*1216V* was also found in Tn*1546* with the *vanA* gene (encoding for vancomycin resistance) in vancomycin-resistant *S. aureus* (VRSA)[38] or in a possible composite transposon containing Tn*1546* in *Enterococcus faecium*.[39] MES_{PM1} most probably originates in enterococci.[5] Occasionally, the Taiwan CA-MRSA clone also manifests tetracycline resistance.[5,10] which is encoded for by the MES_{tet}, located on a penicillinase (PCase) plasmid.[5]

PM1 is a typical example of full multidrug resistance, showing resistance to six non-β-lactam agents (erythromycin, clindamycin, kanamycin, streptomycin, chloramphenicol, and tetracycline),[5] while M013 lacks the MES_{PM1} sequence, containing only IS*1216V* at the chromosomal *att* site (in the *sasK* gene).[17] In terms of drug resistance, M013 is similar to a minor strain (PM18) of the Taiwan CA-MRSA clone, which is susceptible to non-β-lactam agents, and found in approximately 4.2% of Taiwan CA-MRSA isolates.[5] For M013, no information is available for plasmids.[17]

KS1 and PM1 shared the same genetic characteristics, except for SCC*mec* and ampicillin and tetracycline resistance (encoded by a plasmid). As suggested previously,[19] KS1, which lacks the marker genes (*sep* and *sak*) of phage 3, could be an ancestral strain of the Taiwan CA-MRSA clone. Due to the MES_{PM1} structure, KS1, similar to PM1, shows the constitutive phenotype of resistance to clindamycin, a first-line agent for osteomyelitis.

As for virulence factors, those of USA300 have been predicted to be PVL, which disrupts human phagocytes, such as polymorphonuclear neutrophils; colonization factors encoded for by ACME; α-hemolysin (Hla), which plays a role in necrotizing pneumonia; and PSMs (with high expression levels), which play a role in bacteremia and abscess formation.[3]

In terms of virulence factors, KS1 is characterized by PVL, PSMs (with high expression levels), and SEB. PVL was initially noted as a virulence factor of deep skin infections

with large abscesses, such as furuncles and cellulitis,[40,41] and has increasingly been noted in association with osteomyelitis.[33,42] PVL enhances the severity of osteomyelitis in a rabbit model.[43] Moreover, a combination of PVL, protein A, and Coa plays a role in bone loss and bone destruction in osteomyelitis.[44] Moreover, PVL seems to act as an MRSA spread factor, allowing wide transmission through skin-to-skin contact, by the secretion of MRSA in pus on the body surface.[4,45]

SEB (a superantigen) is associated with pediatric osteolyelitis[46] and is the second most prevalent toxin in osteomyelitis.[42] SEB is also an immune evasion factor in staphylococcal infections.[47]

The high expression of PSMs is a common characteristic of CA-MRSA.[2,48] In addition to their possible role as virulence factors as mentioned above, it was proposed that truncated PSMs contribute to CA-MRSA niche establishment on the skin (spread in the population).[48] Indeed, both PVL^+ SCC*mec*V CA-MRSA (Taiwan clone) and Taiwanese PVL^- SCC*mec*IV CA-MRSA expressed the *psm*α and *hld* genes at high levels. CA-MSSA KS1 also exhibited high *psm*α and *hld* gene expression levels.

Since PVL is considered to be a marker of CA-MRSA, we also analyzed the PSM expression levels of other PVL^+ MSSA strains and demonstrated that the expression levels of PVL^+ MSSA strains were significantly higher than those of PVL^- MSSA strains (isolated in hospitals).

Taken together, it is suggested that PVL and PSMs (with high expression levels) contribute to *S. aureus* spread, niche formation, and SSTI (such as furuncles) in the community, and that PVL, PSMs (with high expression levels), and SEB contribute to the pathogenesis of invasive infections, for example, bloodstream infection, osteomyelitis, and pulmonary embolism for KS1. Further studies are needed to clarify the virulence ability, associated with osteomyelitis and pulmonary embolism, of the Taiwan CA-MRSA clone and related ST59 CA-MSSA.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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